Interaction of Hyperthermia and cis-Diamminedichloroplatinum(II) Alone or Combined with Radiation in a C3H Mammary Carcinoma in Vivo

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ABSTRACT

The interaction between hyperthermia and cis-diamminedichloroplatinum(II) (c-DDP) given in various schedules as an adjuvant to radiation treatment was investigated in a C3H mouse mammary carcinoma in vivo. Both hyperthermia (43.5°C for 60 min) and c-DDP (6 mg/kg i.p.) caused a delay in tumor growth when given individually. When c-DDP was given 4 h prior to hyperthermia, the increase in tumor growth time corresponded to an additive effect, but when the interval was reduced to 15 min, the tumor growth delay was significantly greater than additive. The modifying effect of these schedules on radiation was studied using local tumor control (50% tumor control dose) as the endpoint. c-DDP alone did not result in any enhancement of tumor control, irrespective of whether it was given 15 min or 4 h after irradiation. In contrast, heat treatment at 43.5°C for 60 min given 4 h after irradiation resulted in a significant reduction in the 50% tumor control dose, with an enhancement ratio of 1.8. From a clamped local tumor control assay, it was found that c-DDP selectively killed aerobic cells, whereas hyperthermia was primarily directed toward the hypoxic clonogenic cells in the tumors. Combining the two modalities (simultaneously) resulted in a significant additional increase in the killing of well-oxygenated clonogenic cells, but the destruction of hypoxic cells was not different from that obtained after heat alone.

INTRODUCTION

The effect of most chemotherapeutic drugs is known to be enhanced by elevated temperatures (1–4). The mechanisms may be several, including increased drug distribution to the cells by enhanced passive or active transport across the cell membrane; interaction with the mechanism of action of the drugs; effect on drug resistance or several other mechanisms (1–4). In addition, drugs and hyperthermia may act on different tumor cell populations in a complementary way (5–9).

c-DDP is one of the drugs which experimentally has been most intensively investigated, and it has in general been found to be more effective under hyperthermic conditions (10–16). Consequently, this treatment has been introduced clinically, either in association with whole body hyperthermia, or more recently, together with local hyperthermia in the treatment of locally advanced tumors (1–4, 17–21). Since c-DDP in some experimental tumors was found to enhance the effect of radiation (16, 22, 23), the combined heat and c-DDP treatment has also been associated with radiotherapy, so called trimodality therapy (4, 24).

Most experimental studies have been performed in vitro, or in vivo, using growth delay as the end point. Although this may be a suitable model for evaluating an interaction between heat and drug treatment (which together seldom result in tumor control), it may not necessarily reflect the likely outcome when these two modalities are combined with radiotherapy in a curative treatment of locally advanced tumors. In this case, persistent tumor control should be the end point in order to reflect killing of all clonogenic cells. We have previously shown a lack of correlation between growth delay from individual modalities and the ability to modify tumor control induced by radiation (25). In the latter case, reduction in radiation dose would require that the other modalities cause destruction of the most radioresistant (hypoxic) tumor cells. This has been found to be the situation with hyperthermia, whereas in our tumor model c-DDP has no apparent effect on this subpopulation (7). On the other hand, c-DDP has shown a pronounced ability to destroy well-oxygenated and proliferating tumor cells, causing a subsequent prominent reduction in tumor growth (7, 9).

The purpose of the present study was to investigate the interaction between hyperthermia and c-DDP given in various schedules as an adjuvant to radiation treatment of a solid tumor in vivo. The study design allowed evaluation of the individual effect on the subfractions of hypoxic and oxygenated cells, respectively.

MATERIALS AND METHODS

The tumor model and treatment procedure has previously been described in detail (7, 26–28).

Animal Tumor Model

Male or female C3H/Tif/Bom × D2BA/Bom F1 mice were challenged with a mammary carcinoma spontaneously arisen in the C3H/Tif strain. For experimental purposes, the tumors were injected into the foot on the right hind leg. Tumors at a size of approximately 200 mm³ were used for treatment. Tumor volume was measured according to the formula

\[ V = \frac{\pi}{6} \times D1 \times D2 \times D3 \]

where \( D \) represents three orthogonal diameters.

Treatment

Drug. c-DDP (Platinol; obtained by the courtesy of Bristol Myers, Copenhagen, Denmark), was injected i.p. in a concentration of 0.3 mg/ml. In all experiments, a single dose of 6 mg/kg was applied.

Hyperthermia. Heat treatment was applied by placing nonanesthetized animals into a special Lucite jig, allowing the tumor-bearing leg to be immersed in a temperature-controlled circulating water bath (Model PF 923, HETO, Copenhagen, Denmark). The water temperature was adjusted to 43.7°C which resulted in an intratumoral temperature of 43.5°C (26). A total heating time of 60 min was applied in all treatments. The local tumor heating resulted in a slight increase in core body temperature (29). No attempts were made to modify this effect.

Radiation. Local irradiation to the tumor-bearing leg was performed by placing nonanesthetized animals in the same jig as used for hyperthermia. Radiation was performed with a 250 kV Philips X-ray machine at a dose rate of 2.26 Gy/min (10 mA, 3.1 mm Cu half-value layer).
order to secure maximum backscatter, radiation was given with the tumor-bearing leg immersed in water at room temperature.

Irradiation under clamped conditions was performed in a similar way with the exception that the blood supply to the tumor-bearing leg was interrupted by tightening a rubber tube proximal to the tumor 5 min before and during radiation (7).

End Points

The end points for tumor response were tumor growth time, i.e., the time for an individual tumor to increase its volume 5 times. This end point was used for treatments where hyperthermia and c-DDP were given alone or combined.

For studies involving radiation, the end point was TCD50, i.e., the dose which on average results in tumor control in one-half of the treated animals. In these experiments, graded radiation doses were applied to groups of mice in addition to a fixed treatment with hyperthermia and c-DDP or combinations thereof. The TCD50 value was computed by logit analysis (8).

Animals in tumor control studies were observed for 90 days or until definitive recurrence of the tumor.

Based on the TCD50 value for combined treatment and radiation alone, an isoeffect ER was evaluated as:

\[
ER = \frac{TCD_{50}(\text{combined treatment})}{TCD_{50}(\text{radiation only})}
\]

The calculation of absolute cell numbers and hypoxic and oxygenated fractions was performed as previously described in detail by Grau et al. (7, 8). In brief, HF was derived from the TCD50 values obtained from treatments given in air and under clamped conditions, respectively, as:

\[
HF = e^{-D_0(\text{hypoxic})}
\]

In these estimates, a D0 at 3.2 Gy for the irradiation inactivation of C3H mammary carcinoma cells under hypoxic conditions was assumed. The HF following the combined treatments were calculated from the same formula.

The total number of tumor cells were estimated on the basis of the TCD50 for clamped tumors and the radiation sensitivity for hypoxic cells:

\[
\ln N = \frac{TCD_{50}(\text{clamp})}{D_0(\text{hypoxic})} - \ln n + \ln 0.693
\]

where N is the total number of tumor cells; D0(hypoxic) is 3.2 Gy, and n is the extrapolation number, assumed to have a value of 3 (7, 8). Based on the calculations above, the hypoxic and aerobic cell compartments were derived.

The survival of aerobic and hypoxic cells in combined treatment schedules was used to calculate the relative effectiveness of the different adjuvant treatments. The relative survival (SF) was calculated as:

\[
SF = \frac{N(\text{combined treatment})}{N(\text{radiation only})}
\]

RESULTS

Both hyperthermia and c-DDP caused a delay in tumor growth when given individually (Table 1). When applied as a combined treatment, the tumor growth time increased further, depending on the interval between the two treatments. When c-DDP was given 4 h prior to hyperthermia, the increase in tumor growth time corresponded to an additive effect of the two modalities. However, when the interval between c-DDP and hyperthermia was reduced to 15 min, the tumor growth delay was significantly higher than expected from an additive point of view. Thus it appears that the most beneficial treatment schedule to the tumor was a simultaneous application of the two modalities.

In order to investigate how these two different treatment schedules would modify the radiation response, the treatments were combined with graded doses of radiation (Fig. 1). Table 2 shows the TCD50 values for radiation alone or combined with various schedules of hyperthermia and drug. c-DDP given alone after irradiation did not result in any enhancement of tumor control. In contrast, a heat treatment at 43.5°C for 60 min given 4 h after irradiation resulted in a significant reduction in the TCD50, yielding an ER of 1.8. The application of simultaneous hyperthermia and radiation was not performed in the studies because it is known to result in significant sensitization (26) which was not wanted in the present experiments. The application of trimodality therapy was performed in two different schedules, either combining radiotherapy with “sequential” or “simultaneous” drug and heat treatment, i.e., allowing an interval of either 15 min or almost 4 h between c-DDP and hyperthermia. In all trimodality treatment schedules, drug and hyperthermia were applied after radiation, again in order to avoid any direct interaction between radiation and either of the
cells in the tumors. Combining the two modalities (simultaneously) resulted in an additional reduction in the killing of well-oxygenated clonogenic cells, whereas the destruction of hypoxic cells was not different from that obtained after heat alone (Fig. 2).

The hyperthermic enhancement of c-DDP on the tumor effect was unfortunately associated with increased drug toxicity when the two modalities were simultaneously applied. This was expressed by an increased acute lethality as seen in Table 4.

DISCUSSION

The present study has confirmed that hyperthermia is able to enhance the c-DDP-induced growth delay in tumors (3, 10, 11, 14, 30). This enhancement depends on the interval between the two modalities in such a way that a long interval (e.g., 4 h) results in an additive effect, whereas a simultaneous application causes an apparent significant synergism (10, 11, 13, 30). The cause of this effect is unknown, but increased drug uptake through the cell membrane under elevated temperatures has previously been described (1-4) and may be a likely explanation in the present situation.

It is noteworthy that although addition of c-DDP to hyperthermia did not result in an increased killing of hypoxic cells, the overall number of clonogenic cells was reduced by more than 3 orders of magnitude. In the present experiments, given with single doses, it leaves a persistent radioresistant (hypoxic) fraction of cells. However, in a tumor with fewer hypoxic cells or as a part of a more protracted and fractionated treatment schedule allowing reoxygenation (31), the hypoxic problem may eventually be overcome.

The hypoxic cells identified by the current experimental technique are those which are radiobiologically hypoxic (7, 8). They may represent both chronic hypoxic cells in a nutritionally deprived environment as well as transiently hypoxic cells (28, 32). Such acute hypoxia will only last a short period of time and may change significantly within minutes or hours (33). The fact that c-DDP does not affect hypoxic cells is probably not due to the same parameters making the cells resistant to radiotherapy, since acute hypoxia per se may not necessarily influence the drug-induced cytotoxicity (34). However, with the rapid pharmacokinetic turnover of c-DDP in mice (35), even transient hypoxia, due to a brief vascular occlusion at the time of a bolus injection, would prevent these acute hypoxic cells from being subjected to the chemotherapeutic agent. In addition, the proliferative capacity of the chronically hypoxic cells is very low, and the effect of c-DDP on such nonproliferating tumor cells may be minor (4). Combining the current treatment schedule with an agent, such as nicotinamide, directed toward overcoming the acute hypoxia may be beneficial (28, 36, 37).

Since hypoxic tumor cells are the major cause of resistance to radiotherapy and as such demand complete destruction in order to secure tumor control, it is the killing of hypoxic cells...
which determines the TCD50 value (8). The lack of interaction between heat and c-DDP on this fraction explains why the trimodality therapy does not result in better tumor control than the combination of heat and radiation only. On the other hand, hyperthermia enhanced the cytotoxic effect of c-DDP on well-oxygenated and proliferating cells. Thus, the improved effect seen with simultaneous application of heat and c-DDP appeared to be due to a heat-induced sensitization of the drug effect, whereas the drug did not cause any enhancement of the hyperthermic damage. Increasing the interval between the two modalities causes this heat enhancement to disappear, leaving only an additive effect of the two modalities.

The present study indicates that the use of experimental tumor models to evaluate multimodality therapy should be performed with care because the results may depend on the end points used in the study. So far, most interaction studies between hyperthermia and c-DDP have been performed in vitro (3, 12, 13, 15) or by using growth delay in vivo (3, 10, 11, 14). This may overestimate the potential benefit of such combined treatment, especially when applied to solid tumors, which are likely to include a component of nutritionally deprived chronic hypoxic tumor cells (25). On the other hand, if a treatment is given to fairly small tumors with sufficient vascularization and nutrition, a combined schedule may be advantageous. In this case, a simultaneous application of heat and drug apparently will result in the most pronounced tumoricidal effect (11, 13, 30).

The present experiments were designed with special attention to the heat-drug interaction. Although radiation was included in the experiments, the role of this treatment has mainly been in the form of an experimental tool used to obtain tumor control and an estimation of the number of clonogenic cells in situ. For this purpose it has been important to avoid any interaction between radiation and the other modalities. The studies have therefore not taken advantage of any potential radiosensitizing effect which can be induced by both hyperthermia and c-DDP (22, 23, 26). An optimization of the treatment schedules in this regard obviously has the potential of improving the tumor control area, the experimental simulation should apply a

REFERENCES


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